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Herbicides as Ripeners for Sugarcane

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Chemical ripening of sugarcane is an important component to profitable sugar production in the United States as well as other sugarcane industries throughout the world. Harvesting of sugarcane often begins before the sugarcane reaches the desirable maturity level. This is especially true in the Louisiana sugarcane industry where the window for harvesting is limited because of the risk of freezing temperatures encountered in a temperate climate. Research on the application of chemicals, mostly of herbicide origin, to enhance sucrose accumulation (ripening) or limit flowering to conserve stored sucrose has been conducted for more than 60 yr. The only sugarcane ripener currently registered for use in the United States is glyphosate applied before harvest. The herbicide fluazifop is used as the primary ripener of sugarcane in South Africa. The herbicides glyphosate, fluazifop, and sulfometuron-methyl and the growth regulators ethephon and trinexapac-ethyl are registered for use in Brazil. There is a continuing need to evaluate sugarcane ripeners to increase the utility of currently registered ripeners and to find additional ripeners for use by sugarcane industries. The need for alternatives to glyphosate is especially critical before a glyphosate-tolerant sugarcane can be utilized to improve control of problematic weeds.

Nomenclature: Ethephon; fluazifop; glyphosate; sulfometuron-methyl; trinexapac-ethyl; sugarcane, *Saccharum* interspecific hybrid.

Key words: Growth regulators, sucrose, sugar.

Sugarcane is cultivated as a vegetatively propagated perennial crop. In Louisiana, sugarcane is planted in late summer using either whole stalks or stalk pieces that are placed in planting furrows and covered with 5 to 10 cm of soil. Shoots emerge from the axillary buds occurring every 10 to 15 cm along the sugarcane stalk with tillers forming from each shoot. Growth of the sugarcane is limited during the winter months but increases with the warmer temperatures of spring and summer when stalk height can increase 2 to 3 cm each day. This first year's crop, termed the plant-cane crop, is generally harvested in November or December. After harvest, crop residue is removed by burning and shoots emerge from underground buds to form a ratoon crop. Normally, two or three ratoon crops are harvested annually during the harvest season, which lasts from late September through early January, before yield declines require that fields be replanted.

Sugarcane's value is determined by the amount of recoverable sugar per weight of cane and is the basis by which sugarcane farmers in Louisiana are paid. Theoretically recoverable sugar (TRS) is determined at the mill by taking a core sample from trucks as they are delivering their cane. Samples are ground and crushed to extract juice that is then tested for Brix (percentage soluble solids by weight) using a refractometer, Pol (percentage apparent sucrose by weight) using a saccharimeter, percentage fiber of the cane, and percentage sediments to determine TRS (kg sugar/Mg cane). Commercially recoverable sugar is a percentage of TRS determined by the mill according to their efficiency in extracting sugar and is typically around 95% of TRS.

Sugarcane varieties with high sugar content are more economical for mills to process than high-biomass varieties with lower sugar content. Unfortunately, the relatively short growing season for sugarcane in Louisiana, compared with most other geographic locations where sugarcane is grown, requires that the Louisiana harvest season begin in late September or early October when the sucrose content in sugarcane is low. This need for an early harvest start is exacerbated by the decrease in the number of sugar mills and the increased productivity of sugarcane growers hauling greater quantities of cane. In 1964, there were 48 operating sugar mills in Louisiana; for the 2008 harvest there were 11 mills. In 1964, sugarcane growers produced 7.1 million Mg of cane on 140,000 ha (USDA National Agriculture Statistics Service 2009). In 2008, sugarcane growers produced 10.7 million Mg of cane on 164,000 ha. This increase in production and decrease in mills has not only required mills to increase capacity and to become more efficient, but has also required an increase in the length of the harvest season.

In Louisiana, harvest season ends in late December or early January to reduce the risk of the crop being exposed to freezing temperatures that can cause the cane to deteriorate within a few weeks to a point where economical sugar recovery is no longer viable (Eggleston et al. 2004). As the end of the harvest season cannot be extended, this requires that the harvest season begins earlier. This comes with a cost in profitability in that TRS levels are lower at the beginning of the harvest season. Additionally, harvesting early has been shown to reduce the yield potential of the subsequent ratoon crop (Viator et al. 2009).

Sugarcane maturation or ripening is evident from the accumulation of sucrose in the internodes. This is stimulated by several environmental cues including cooling temperatures, high daily incident sunlight, low soil moisture, and low soil nutrient content (Legendre 1975), none of which can be managed in Louisiana's rain-fed production systems. As harvest constraints prohibit sugarcane from maturing naturally, it has become necessary to evaluate and develop chemicals, mostly of herbicide origin, to induce ripening early in the harvest season.

History of Ripener Evaluation

Potential sugarcane ripeners have been evaluated at the U.S. Department of Agriculture–Agricultural Research Service (USDA-ARS) Sugarcane Research Lab since 1948 (Legendre 1974). Over the years, more than 100 different chemicals have been tested for their potential to ripen sugarcane. Many of the

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chemicals are tested for their ability to enhance sucrose concentration in sugarcane are herbicides, whereas others are nutrients or hormonal plant growth regulators. Most of these chemicals failed to provide a consistent ripening effect on sugarcane in Louisiana. The first chemical showing real potential for ripening sugarcane in the United States was glyphosine [*N*,*N*-bisphosphonomethyl glycine], which was registered in the United States as a chemical ripener of sugarcane on a limited basis in 1972 and gained a full registration in 1975 (Frost 1976; Legendre 1974; Legendre and Martin 1977).

Glyphosine was used on a limited basis in Louisiana but was applied to more than 18,000 ha in Florida in 1979 (Dusky et al. 1986). In Hawaii, glyphosine was used on around 65% of sugarcane harvested in 1979 (Hilton et al. 1980). However, the response of sugarcane to glyphosine was inconsistent and some varieties failed to respond (Dusky et al. 1986; Hilton et al. 1980). Soon after the commercial use of glyphosine was adopted, a new compound, glyphosate, was introduced for testing as a ripener, beginning in 1978 (EPA 1978). Then in 1980, glyphosate was registered for use as a growth regulator in sugarcane (EPA 1980). Glyphosate was shown to be more consistent at ripening sugarcane, with fewer differences in varietal response and a lower use rate that led to the discontinuation of the use of glyphosine after 1985 (Dusky et al. 1986; Hilton et al. 1980; Watson and DeStefano 1986). However, even with glyphosate, response has been shown to be dependent on variety (Millhollon and Legendre 2000). This varietal difference has been reported not only in the resulting sugar content of the harvested cane, but also in the injury to the subsequent ratoon crop that emerges in the spring after a glyphosate application to the previous crop (Millhollon and Legendre 1996).

Today, glyphosate is the only sugarcane ripener registered for use in sugarcane in the United States. Glyphosate has been shown to increase recoverable sugar, but sugar yields do not always increase, as reductions in apical growth lower gross cane yields in comparison with nonripened cane (Clowes 1980). Millhollon and Legendre (1996) showed that applying glyphosate for three consecutive years reduced total cane yield by 4% but increased sugar yield by 7%. The increase in sugar yield was due to a 12% average increase in TRS. Reducing total cane yield while increasing sugar yield is economically advantageous to both the farmer and the mill. Since sugarcane farmers in Louisiana are paid by the amount of sugar present in the cane that is delivered (typically a 60 : 40 split between the farmer and mill), reducing the cane tonnage reduces transportation costs and the higher sugar content means more sugar is delivered with each load.

Ripeners are routinely used in other sugarcane industries throughout the world. In Brazil, glyphosate, ethephon [2-chloroethylphosphonic acid], fluazifop, sulfometuron, and trinexapac-ethyl [ethyl 4-(cyclopropylhydroxymethylene)-3,5-dioxocyclohexanecarboxylate] are used not only as ripeners but to suppress flowering (de Almeida et al. 2003; Caputo et al. 2007; Guimarães et al. 2005; Leite and Crusciol 2008). Flowering, which can reduce sugar content, is not a concern in the Louisiana sugarcane industry as climatic conditions do not normally induce flowering. In South Africa, ethephon, fluazifop, glyphosate, and glyphosine have all been registered for use as sugarcane ripeners but only fluazifop is currently used (Donaldson 1999; Donaldson and Van Staden 1989). In Australia, the potential utility of glyphosate, fluazifop, and ethephon as sugarcane ripeners is being explored (Morgan et al. 2007).

Ethephon is a growth regulator used to promote ripening of fruit and tobacco, as well as sugarcane. After absorption into plant tissues it rapidly converts into ethylene, phosphate, and a chloride ion (Yang 1969). This promotion of ethylene in plants is responsible for the ripening effect. In sugarcane, ethephon is used both to advance ripening and to inhibit flowering (Caputo et al. 2007; Li and Solomon 2003). The exact mechanism by which ethylene acts upon sugarcane has not been elucidated.

Fluazifop is an aryloxyphenoxy propionate herbicide that inhibits acetyl-coenzyme A carboxylase (ACCase), an enzyme involved in fatty acid biosynthesis (Burton et al. 1989). Disruption of ACCase inhibits the formation of lipid membranes needed for cellular growth, resulting in the death of meristematic tissues, particularly in the stalk apex (Donaldson and Van Staden 1995). The effects of fluazifop on sugarcane are slow acting and do not directly interrupt photosynthesis, allowing for continued sucrose accumulation while terminal growth ceases. Watson and Stefano (1986) showed fluazifop at 0.13 to 0.28 kg ai ha^{-1} to be as effective at ripening sugarcane as glyphosate in Florida. In Louisiana, fluazifop at 0.28 kg ai ha^{-1} increased sucrose in six of seven sugarcane varieties but was less efficient than glyphosate because of increased losses in stalk weight in fluazifop treatments (Watson and Stefano 1986). Although fluazifop is the primary ripener used in the South Africa sugarcane industry, it has not been registered as a ripener for sugarcane in the United States.

Trinexapac-ethyl, an acylcyclohexanedione, inhibits growth through disruption of gibberillic acid biosynthesis by blocking the 3β-hydroxylase enzyme that reduces cell elongation (Nakayama et al. 1990; Rademacher 2000). It is used primarily in turfgrass to reduce mowing requirements and in grass seed production to prevent lodging and reduce needs for burning (Johnson 1992; Zapiola et al. 2006). In sugarcane, studies have shown that trinexapac-ethyl can increase sucrose levels while having a minimal effect on stalk weight and regrowth (Guimarães et al. 2005; Rainbolt 2005a; Richard et al. 2006). Trinexapac-ethyl is used extensively as a ripener in Brazil and studies on its potential for use as a ripener for sugarcane in the United States are continuing (Guimarães et al. 2005; Resende et al. 2000).

Glyphosate inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase in nearly all plants. This halts the conversion of shikimate-3-phosphate into anthranilic acid (chorismate), the precursor to the production of the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Amrhein et al. 1980; Hollander and Amrhein 1980; Jaworski 1972; Steinrucken and Amrhein 1980). Even though glyphosate is used primarily as an herbicide worldwide, and the primary site of action is known, it is still somewhat unclear as to how plants exposed to glyphosate are killed. Although it would be convenient to assume that the inability of plants to produce aromatic amino acids necessary for protein assimilation is the cause, others suggest that the cause is the deregulation of the pathway itself, causing massive accumulation of shikimate and the loss of carbon for other pathways, including the reactivation of ribulose bisphosphate carboxylase, a key enzyme in photosynthesis, and that this ultimately leads to plant death (Servaites et al. 1987; Siehl 1997).

For the ripening of sugarcane, glyphosate is applied at sublethal doses, 0.16 to 0.47 kg ae ha^{-1}. This application retards vegetative growth and stimulates sucrose accumulation (Legendre and Finger 1987). In effect, glyphosate hastens the ripening of sugarcane by inhibiting growth of the apical meristem while enzymes involved in photosynthesis and in the transportation and storage of sucrose remain uninhibited (Lingle 1999; Maretzki et al. 1976; Nomura et al. 1986; Su et al. 1992). Nomura et al. (1986) showed that stalk growth ceased at 10 d after glyphosate application, and death of the sugarcane spindle, the cylinder of several concentric whorls of tightly packed elongating leaves positioned above the shoot apical meristem (Meinzer and Moore 1988), occurred at 2 to 3 wk. Su et al. (1992) showed a sevenfold increase in sucrose content in the immature and rapidly elongating fifth internode 5 d after glyphosate treatment, whereas little accumulation occurred in the controls. They also showed that glyphosate did not affect the activity of the enzymes responsible for sucrose metabolism, specifically sucrose P synthase, sucrose synthase, and neutral invertase, but did cause an 80% reduction in the activity of acid invertase.

Acid invertase, which catalyzes the hydrolysis of sucrose into glucose and fructose, is typically high in elongating immature sugarcane internodes. However, reduction in this enzyme alone was shown to not be entirely responsible for increased sucrose levels in glyphosate-treated internodes as removal of the apical meristem also reduced acid invertase without a responding increase in sucrose accumulation. Most likely, increased sucrose accumulation was due to an altered partitioning of carbohydrates as growth was inhibited after glyphosate application, resulting in carbohydrates produced through photosynthesis being stored rather than utilized for growth.

Glyphosate use is restricted to the ratoon cane crops in Louisiana and Texas (Millhollon and Legendre 1996) and only on the final ratoon crop in Florida (Dusky et al. 1986), as there is concern that applications may reduce productivity of subsequent ratoon crops. Millhollon and Legendre (1996) showed that some sugarcane varieties tolerated consecutive glyphosate applications starting in the plant-cane crop, whereas others were less tolerant. Glyphosate injury is often manifested in the springtime as stunted growth with bleach-streaked leaves. In Louisiana, this injury is usually transient and reductions in cane populations and yield are rarely seen except when higher rates of glyphosate (< 0.45 kg ha^{-1}) are used and harvest is delayed beyond 49 d after treatment (Legendre et al. 1980). However, in Florida, more severe reductions in yield and stand losses have been reported (Rice et al. 1984).

When glyphosate was first registered for use as a sugarcane ripener in Louisiana, its designated use was for the last ratoon crop of sugarcane that is generally harvested at the beginning of the harvest season. However, over the years, applications were expanded to include nearly all sugarcane harvested. Most research on the effectiveness of glyphosate ripener applications has been conducted on sugarcane harvested early in the harvest season. Little research had been done to validate the efficacy of applications made late in the harvest season. Recent research at the USDA-ARS Sugarcane Research Lab has shown that the greatest ripening response to glyphosate, as much as a 39% increase in TRS, occurred with applications made in August or September to the cultivar 'HoCP 96-540'. A moderate increase, a 6 to 15% increase in recoverable sugar, was observed when applications were made in the beginning of October. However, there was no increase in sugar content when applications were made the first week of November (E. Richard, unpublished data). These results showed that the greatest value for ripening sugarcane is when the cane is immature and actively growing. When environmental conditions favor natural ripening, the value of ripener applications decreases substantially.

Not all cultivars currently planted in Louisiana respond equally to ripener application rates of glyphosate. However, increasing the rate of glyphosate application on 'L97-128', a poor-responding variety, did not increase sucrose content compared with typical application rates. But when glyphosate rates were increased, it caused a loss in cane and sugar yield in the subsequent ratoon crop (E. Richard, unpublished data).

Research for Alternative Ripeners

Research aimed at finding additional ripeners is needed for several reasons. First, glyphosate is not registered for use on plant cane in Louisiana and can only be used on the final ratoon in Florida (Dusky et al. 1986). Thus, much of the sugarcane cannot be treated with a ripener before harvest. Second, the injury that glyphosate causes to the emerging ratoon crop the following spring is a concern for many growers who are deciding which fields they should keep and which should be destroyed and replanted. Glyphosate often delays spring growth and causes emerging shoots to be bleached and stunted, making them less competitive with emerging weeds such as bermudagrass [*Cynodon dactylon* (L.) Pers.], and makes the decision on whether to keep or destroy a field more difficult. Third, the potential development of glyphosate-tolerant sugarcane would be impeded by lack of an effective sugarcane ripener, as glyphosate would no longer have efficacy as a ripener. Glyphosate-tolerant sugarcane would be valuable to the sugarcane industry as a means of controlling perennial grass species, such as bermudagrass and johnsongrass [*Sorghum halepense* (L.) Pers.] in Louisiana, and napiergrass (*Pennisetum purpureum* Schumach.) in Florida (Rainbolt 2005b).

Research on the efficacy of sugarcane ripeners is usually conducted in small plots with treatments being applied with a handheld overhead spray boom. These treatments are applied 4 to 8 wk before a planned harvest date. Treatments are normally applied in late August or September and can only be applied while cane is erect. If canes lodge before treatment application, tests must be abandoned. Hand-cut samples are used to evaluate the efficacy of ripener treatments and consist of 10 to 15 stalks from a random location within each plot. Samples are cut weekly typically beginning at 4 wk after ripener application. Samples are weighed to determine average stalk weight and then are processed to determine sucrose concentration by passing them through a roller mill to express juice or by finely chopping the cane and placing a subsample into a hydraulic press to express juice. Juice samples are tested for Brix and Pol. The remaining cane stalks in each plot are then mechanically harvested using a chopper harvester at 7 or 8 wk after treatment application to determine cane yield with samples of the harvested stalk pieces (billets) removed to assay for quality as described previously.

Current research into alternative ripeners has focused on the gibberellin inhibitor trinexapac-ethyl, and on the

acetolactate synthase inhibitors imazapyr and nicosulfuron. Richard et al. (2006) reported an increase in theoretically recoverable sugar in the sugarcane 'LCP 85-384' treated with glyphosate at 0.2 kg ae ha^{-1}, trinexapac-ethyl at 0.3 kg ai ha^{-1}, imazapyr at 0.1 kg ai ha^{-1}, and nicosulfuron at 0.05 kg ai ha^{-1} when measured at 6 and 7 wk after treatment compared with a nontreated control. Sugar yields were also increased for the trinexapac-ethyl, imazapyr, and nicosulfuron treatments; however, there was no increase in sugar yield for the glyphosate treatment because of reductions in cane yield.

For additional sugarcane ripeners to gain registration, they must consistently increase sucrose levels, similarly to what is found with glyphosate treatments. Additionally, they must be less injurious to the subsequent ratoon crop than glyphosate. Testing is being conducted on sugarcane varieties recommended for planting in Louisiana. Positive results across several varieties would be the first step in the pursuit of registration of these chemicals for use as ripeners for sugarcane.

Chemical ripening of sugarcane plays an important role in the U.S. sugarcane industry as well as in sugarcane industries around the world. The ability to hasten maturity allows for increased economical recovery of sugar that benefits both the grower and the mill. Research will continue to improve recommendations for registered ripeners and aid in the search for additional ripeners.

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